

RONNY LARSSON

**Studies of the Cytology and
Taxonomy of the Microsporidia
(Protozoa, Microspora)**



Department of Zoology
University of Lund, Sweden
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STUDIES OF THE CYTOLOGY AND TAXONOMY OF THE MICROSPORIDIA
(PROTOZOA, MICROSPORA)

RONNY LARSSON

FM, Ld

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LIST OF PAPERS

The thesis is based on the following papers:

- I Larsson, R. (1980). - An ultrastructural study of Toxoglugea variabilis n. sp. (Microsporidia, Thelohaniidae), a microsporidian parasite of the biting midge Bezzia sp. (Diptera, Ceratopogonidae). Protistologica 16: 17-32.
- II Larsson, R. (1980). - Insect pathological investigations on Swedish Thysanura. II. A new microsporidian parasite of Petrobius brevistylis (Microcoryphia, Machilidae); Description of the species and creation of two new genera and a new family. Protistologica 16: 85-101.
- III Larsson, R. (1981). - The ultrastructure of the spore and the sporogonic stages of Telomyxa glugeiformis Léger and Hesse, 1910 (Microsporidia, Telomyxidae). Zool. Anz. 206: 137-153.
- IV Larsson, R. (1981). - A new microsporidium Berwaldia singularis gen. et sp. nov. from Daphnia pulex and a survey of microsporidia described from Cladocera. Parasitology 83: 325-342, Pl. I-IV.
- V Larsson, R. (1981). - Description of Nosema tractabile n. sp. (Microspora, Nosematidae), a parasite of the leech Helobdella stagnalis (L.) (Hirudinea, Glossiphoniidae). Protistologica 17: 407-422.
- VI Larsson, R. (1981). - An ultrastructural study of Amblyospora undulata n. sp., a microsporidian parasite of the caddisfly Cyrnus trimaculatus (Trichoptera, Polycentropidae). Protistologica 17: 511-523.
- VII Larsson, R. (1982). - Thelohania capillata n. sp. (Microspora, Thelohaniidae) - An ultrastructural study with remarks on the taxonomy of the genus Thelohania Henneguy, 1892. Arch. Protistenk. 126 (in press).
- VIII Larsson, R. (1982). - Cytology and taxonomy of Helmichia aggregata gen. et sp. nov. (Microspora, Thelohaniidae), a parasite of Endochironomus larvae (Diptera, Chironomidae). Protistologica 18: 355-370.
- IX Larsson, R. (1983). - On two microsporidia of the amphipod Rivulogammarus pulex - Light microscopical and ultrastructural observations on Thelohania muelleri (Pfeiffer, 1895) and Nosema rivulogammari n. sp. (Microspora, Thelohaniidae and Nosematidae). Zool. Anz. (in press).
- X Larsson, R. (1983). - A revisionary study of the taxon Tuzetia Maurand, Fize, Fenwick and Michel, 1971, and related forms (Microspora, Tuzetiidae). Protistologica (in press).

The papers will be referred to by their Roman numerals. A reference to a page is given as a Roman numeral followed by an Arabic numeral. Figures are referred to by Fig. followed by their Arabic numerals.



PREFACE

The microsporidia are minute spore-forming parasitic protozoa, intracellular in all stages of development. Some are specific to their hosts, while others can infect a wider host spectrum. Suitable hosts are found in the entire Animal Kingdom, from protozoa to man. Depending on the tissue selected for development, they are more or less harmful. Many species are lethal to their hosts.

The thick-walled spores have a unique extrusion apparatus, composed of a coiled telescopically constructed tube, the polar filament, a lamellar polaroplast, and a posterior vacuole. In response to stimuli of the host the filament is ejected, and after penetration of the cell wall by the filament, the sporoplasm is injected.

Nosema bombycis was the first microsporidium described, although the parasite of the silkworm reported by NAEGELI in 1857 was believed to be a fungus. Descriptions of new species have appeared successively, and today there are approximately 700 valid species. The biology and taxonomy of the group have been treated in important monographies (AUERBACH, 1910; KUDO, 1924; COMPARATIVE PATHOBIOLOGY I-II, 1976-1977), and in numerous papers from nearly all parts of the world.

The occurrence of microsporidia in Scandinavia is practically unknown. The few reports published have dealt with the biology and therapy of Nosema apis and Thelohania contejeani, both parasites of economically important hosts. The only important contribution to microsporidiology from Scandinavia is a thesis on Encephalitozoon cuniculi by PETRI (1969).

The present thesis, consisting of 10 papers, deals with the cytology and taxonomy of 19 microsporidian species, including descriptions of new taxa on species, genus and family level. The summarized results of the papers are included in a brief general survey of microsporidian systematics and the variability of morphological characters, histopathology, and developmental cycles.

THE SYSTEMATIC POSITION OF THE MICROSPORIDIA

At the end of the 19th century the classification of the protozoa, based on organelles of locomotion, was developed - a system, which with small modifications was to survive until electron microscopy provided new types of taxonomical characters. The class Sporozoa LEUCKART, 1879, was treated by LABBÉ (1899) in "Das Tierreich", where it was divided into two "légions": Cytosporidia LABBÉ, 1894, and Myxosporidia BUTSCHLI, 1880. Microsporidia BALBIANI, 1882, was considered as an order of the Myxosporidia, and it comprised the single family Nosematidae, with three genera, and 9 valid and 44 dubious species. DOFLEIN (1901) collected the myxosporidia and the microsporidia in the new order Cnidosporidia, an arrangement that should last for nearly 80 years. The Cnidosporidia was raised to subclass level by POISSON (1953) in "Traité de Zoologie", and it was considered as a class by KUDO (1966). In both these systems the Microsporidia is an order.

The Society of Protozoologists established in 1954 a Committee on Taxonomy and Taxonomic Problems, which 10 years later presented a revised classification of the Protozoa, now raised to a phylum (HONIGBERG et al., 1964). Myxosporidia and microsporidia were still combined as a single unit, subphylum Cnidospora DOFLEIN, 1901, divided into the two classes Myxosporidea BUTSCHLI, 1881, and Microsporidea CORLISS and LEVINE, 1963. The connection between myxosporidia and microsporidia was definitely broken in 1977 when two new classifications of microsporidia were proposed (SPRAGUE; WEISER), and in both systems the microsporidia were treated as a phylum. The Committee on Systematics and Evolution, established by the Society of Protozoologists, published a new protozoological classification in 1980 (LEVINE et al.), which differs strongly from the conventional system. The Protozoa is here a subkingdom, divided into 7 phyla, one of these is the Microspora SPRAGUE, 1977.

THE CLASSIFICATION OF THE MICROSPORIDIA

There have been several attempts to classify the microsporidia. The older contributions are treated by SPRAGUE (1977). The presentation here will be restricted to a few older classifications of principal interest, to a brief comparison of the two newly proposed systems, by SPRAGUE (1977) and by WEISER (1977), and to the new taxa of this thesis.

The system by STEPELL (1909) (Table 1) recognized three families, which were defined in a nearly modern way. The characters used were taken from the development and histopathology: stages with single nuclei were compared to multinucleate plasmodia, and encystment was used as a taxonomical character.

Although the system by LEGER and HESSE (1922) (Table 1) was based on more superficial characters it was conserved for many years, and it was still in use in the system proposed by HONIGBERG et al. (1964). The division into two suborders was based on a misinterpretation. Telomyxa glugeiformis (III) has a peculiar arrangement of the spores. They are glued together pair-wise, and embedded in a cementing substance to bodies of an oval shape. The oval body was interpreted as a spore with two polar capsules. CODREANU (1961) made the correct interpretation. LEGER and HESSE grouped the microsporidia with a single polar filament as one suborder, and T. glugeiformis which appeared to have double polar capsules was the single species of the other suborder. The families were defined by the shape of the spores.

In 1971 the sporophorous vesicle, called pansporoblast, was introduced as a taxonomical character at the suborder level (TUZET, MAURAND, FIZE, MICHEL and FENWICK, 1971) (Table 1). One suborder contained the four families devoid of pansporoblasts. These had usually binucleate sporoblasts. The three families with pansporoblastic sporogony had always uninucleate sporoblasts, and these were grouped together in the other suborder. When, some years later, it was revealed that there are microsporidian genera (Eurenella, Vairimorpha, Amblyospora, Parathelohania) with two principally different sporogonial sequences, it was clear that the classification of the microsporidia still was an unsolved problem.

TABLE 1. Three historical classifications of the microsporidia

STEMPELL (1909)

Order MICROSPORIDIA

- Fam. Nosematidae
- Plistophoridae
- Glugeidae

LEGER and HESSE (1922)

Order MICROSPORIDIA

Suborder Dicnidea

- Fam. Telomyxidae

Suborder Monocnidea

- Fam. Glugeidae
- Cocconemidae
- Krazekiidae

TUZET, MAURAND, FIZE, MICHEL and FENWICK (1971)

Class MICROSPORIDEA

Order MICROSPORIDA

Suborder Apansporoblastina

- Fam. Caudosporidae
- Nosematidae
- Metchnikovellidae
- Mrazekidae

Suborder Pansporoblastina

- Fam. Monosporidae
- Telomyxidae
- Polysporidae

TABLE 2. The classifications of the microsporidia by WEISER (1977) and by SPRAGUE (1977)

Phylum MICROSPORIDIA	from WEISER (1977)			from SPRAGUE (1977)		
CLASSES	Orders	FAMILIES	Genera	FAMILIES	Orders	CLASSES
METCHNIKOVELLIDA	Metchnikovellida	METCHNIKOVELLIDAE	Metchnikovella (+ Amphiamblys) Amphiacantha	METCHNIKOVELLIDAE	Metchnikovella Amphiamblys Amphiacantha	RUDIMICROSPOREA
	Chytridiopsida	CHYTRIDIOPSIDAE	Chytridiopsis (+ Steinhausia) (+ Burkea)	CHYTRIDIOPSIDAE	Chytridiopsis Steinhausia Burkea	CHYTRIDIOPSIDA
	Hesseida	HESSEIDAE	Hessea	HESSEIDAE	Hessea	diopsida
	Pleistophorida	AMELYOSPORIDAE	Amblyospora Chapmanium Hyalinocysta Parathelohania	THELOHANIIDAE	Amblyospora Chapmanium Hyalinocysta Parathelohania Agmasoma 1) Cryptosporina Heterosporis 1) Inodosporus Pegmatheca Pilosporella 1) Systemostrema Thelohania Toxoglugea (+ Spiroglugea) Duboscqia Trichoduboscqia Gurleya Pyrotheca 5) Stempellia Telomyxa Vavraia 2)	Microsporida
MICROSPORIDIDEA		THELOHANIIDAE				MICROSPOREA

Suborder PANSPORELLINA

		Suborder APANS POROBLASTINA	
	Pleistophora	Nitoplistophora	PLEISTOPHORIDAE
	Tuzetia	Pseudopleistophora 1)	PSEUDOPLEISTOPHORIDAE
	Glugea	Tuzetia	TUZETIIDAE
		Encephalitozoon	GLUGEIDAE
	Perezia (+Encephalitozoon)	Glugea	UNIKARYONIDAE
		Spraguea 1)	
		Nosemoides 1)	
		Perezia	
	Weiseria 3)	Unikaryon 1)	COUGOURDELLIDAE
	Cougourdella		
	Culicospora 4)		
	Culicosporella 4)		
Nosematidida	Hazardia 4)		CAUDOSPORIDAE
	Pyrotheca 5)		
	Golbergia 6)	Weiseria 3)	
	Caudosporea	Caudosporea	
	Octosporea	Octosporea	NOSEMATIDAE
		Ameson 1)	
		Ichthyosporidium 1)	
	Issia 7)		
	Nosema	Nosema	MRAZEKIIDAE
	Variimorpha 4)		
	Jirovecia 8)		
	Mrazekia	Mrazekia	
		Microsporidium 4)	

Explanations: 1) excluded by WEISER due to insufficient information

2) Pleistophora in SPRAGUE

3) Weiseria in different positions

4) Microsporidium in SPRAGUE

5) Pyrotheca in different positions

6) Weiseria in SPRAGUE

7) Telomyxa in SPRAGUE

8) Mrazekia in SPRAGUE

TABLE 3. The classification of the microsporidia according to SPRAGUE (1982), with additions of later described genera

Class RUDIMICROSPOREA			
Order Metchnikovellida			
Family METCHNIKOVELLIDAE		Amphiacantha	
		Amphiamblys	
		Metchnikovella	
Class MICROSPOREA			
Order Minisporida			
HESSEIDAE	Hessea	BURKEIDAE	Burkea
CHYTRIDIOPSISIDAE	Chytridiopsis	BUXTEHUDEIDAE	Buxtehudea (II)
	Steinhausia		Jiroveciana (II)
Order Microsporida			
Suborder PANSPOROBLASTINA		Suborder APANSPOROBLASTINA	
PLEISTOPHORIDAE	Mitoplastophora	GLUGEIDAE	Baculea
	Pleistophora		Encephalitozoon
	Vavraia		Glugea
PSEUDOPL.-IDAE	Pseudopleistophora		Loma
DUBOSQCIIIDAE	Duboscqia		Oligosporidium
	Trichoduboscqia	SPRAGUIDAE	Spraguea
THELOHANIIDAE	Agmasoma	UNIKARYONIDAE	Nosemoides
	Chapmanium		Pleistopsporidium
	Cryptosporina		Unikaryon
	Helmichia (VIII)	PEREZIIDAE	Ameson
	Heterosporus		Perezia
	Inodosporus	COUGOURDELLIDAE	Cougourdella
	Ormieresia	CAUDOSPORIDAE	Caudospora
	Pegmatheca		Culicosporella
	Pilospora		Golbergia
	Systemostrema		Octosporea (VIII)
	Thelohania (VII, IX)		Weiseria
	Toxoglugea (I)	NOSEMATIDAE	Ichthyosporidium
BURENELLIDAE	Burenella		Issia
	Vairimorpha		Nosema (V, IX)
AMBLYOSPORIDAE	Amblyospora (VI)	MRAZEKIIDAE	Jirovecia
	Hyalinocysta		Mrazekia
	Parathelohania		
CULICOSPORIDAE	Culicospora		
	Hazardia		
GURLEYIDAE	Gurleya		
	Pyrotheca		
	Stempellia		
TELOMYXIDAE	Berwaldia (IV)		
	Telomyxa (III)		
TUZETIIDAE	Alfvenia (X)		
	Janacekia (X)		
	Nelliemelba (X)		
	Tuzetia (X)		
UNCERTAIN POSITION		UNCERTAIN POSITION	
	Auraspora		Geusia
	Neoperezia		
	Polydispyrenia		

In 1977 there were two new attempts to classify the microsporidia (SPRAGUE; WEISER). The two systems are compared in Table 2. To facilitate comparison some families and genera are listed in a sequence different from the originally published. In both systems new taxa are described. Some insufficiently known genera were excluded by WEISER, and SPRAGUE introduced the collective and unclassified genus Microsporidium for species so incompletely known that they could not be ranked into a genus. SPRAGUE retained Pansporoblastina and Apansporoblastina. WEISER did not use these categories, but considered all microsporidia to have pansporoblasts, although sometimes not persistent. Apart from the different use of the pansporoblast membrane, the two systems differ principally in the treatment of the primitive microsporidia and the delimitation of families. The system by SPRAGUE recognises more families. The definitions of higher taxa cannot be applied strictly in neither of the systems. In WEISER's system the dimorphic microsporidia of Amblyosporidae, which have diplokaryotic free spores, were included in the order Pleistophorida, characterized by uninucleate sporogony and spores, while Vairimorpha, with uninucleate octospores, is found in the order Nosematidida, which has diplokaryotic spores and sporogony. In the system by SPRAGUE Amblyospora and Parathelohania were included in the Pansporoblastina, although they also have a sequence with free spores. In the new classification of the Protozoa proposed by LEVINE et al. (1980) the system by SPRAGUE is followed.

A great problem in the taxonomical treatment of the microsporidia is the difficulty to compare old and new descriptions, since they are often based on different types of characteristics. New types of characters which might be of taxonomical importance can seldom be evaluated in an old material. There are also some recently published new genera where the descriptions are nearly devoid of adequate information. Although possibly all microsporidiologists agree that the present systems are inadequate, there are still so many unsolved problems that it seems immature to propose a radically different system. SPRAGUE (1982) published a revised version of his classification, with additions of newly described genera. This system is found in Table 3, where genera which have appeared afterwards, or, if the descriptions are parts of this thesis, are in print, are included. The genera treated in the thesis are indicated by Roman numerals referring to the papers. In addition a dichotomous identification key to the genera is enclosed as Appendix I.

In the papers of the thesis one new family, 7 new genera, and 13 new species are described. The new taxa are listed below in an alphabetical order, with references to the pages where the descriptions are found.

<u>Alfvenia</u> gen. n. (X: 18)	<u>Janacekia</u> gen. n. (X: 20)
<u>A. nuda</u> sp. n. (X: 18)	<u>J. undinarum</u> sp. n. (X: 23)
<u>Amblyospora undulata</u> sp. n. (VI: 522)	<u>Jiroveciana</u> gen. n. (II: 100)
<u>Berwaldia</u> gen. n. (IV: 338)	<u>Nelliemelba</u> gen. n. (X: 24)
<u>B. singularis</u> sp. n. (IV: 338)	<u>Nosema rivulogammari</u> sp. n. (IX: 15)
<u>Buxtehudea</u> gen. n. (II: 100)	<u>N. tractabile</u> n. sp. (V: 417)
<u>B. scaniae</u> sp. n. (II: 100)	<u>Thelohania capillata</u> sp. n. (VII: 43)
<u>Buxtehudeidae</u> fam. n. (II: 100)	<u>Toxoglugea variabilis</u> sp. n. (I: 27)
<u>Helmichia</u> gen. n. (VIII: 368)	<u>Tuzetia skuldae</u> sp. n. (X: 13)
<u>H. aggregata</u> sp. n. (VIII: 368)	<u>T. urdae</u> sp. n. (X: 14)
	<u>T. verdandiae</u> sp. n. (X: 16)

DIVERSITY AND VARIABILITY OF THE MORPHOLOGICAL CHARACTERS

The shape and size of the spores

Most microsporidia have spores of an oval or pyriform shape. Oval spores are e.g. typical of the genus Nosema (V: Fig. 5), and pyriform of Tuzetia (X: Figs. 1 A-D). The oval spores of Amblyospora (VI: Figs. 3 D-E) are compressed at the poles to a barrel-shape. Spherical spores are typical of the primitive microsporidia of the genera Metchnikovella, Amphiacantha, Hessea, Burkea, Chytridiopsis, Steinhausia, Buxtehudea (II: Figs. 14-15), and Jiroveciana (II: Fig. 19), but also found in one genus of the family Thelohaniidae: Filospora. Some genera have spores of a unique shape. The spores of Heiseria, Bolbergia and Parathelohania are oval with ridges, depressions or collar-like prolongations, characteristic for each genus. The oval spores of Caudospora, and the rod-shaped of Jirovecia, have caudal appendages. The oval spores of Inodosporus have 4-5 filamentous appendages. The spores of Gurleya and Pyrotheca are pyriform, with a pointed anterior end. The rod-shaped spores of Cougourdella have a terminal swelling.

Rod-shaped spores are found in genera of different systematic positions: in Amphiamblys of the Metchnikovellida, in Ormieresia and Helmichia (VIII: Figs. 3-5) of the Pansporoblastina, and in Baculea, Nosemoides, Octospora and Mrazekia of the Apansporoblastina. Bent, rod-shaped spores are characteristic of Toxoglugea (I: Figs. 9-11). SPRAGUE (1977) collected the microsporidia with bent or spiralized rod-shaped spores, which previously had been treated as several genera, in the genus Toxoglugea. The morphological varia-

tion found in T. variabilis (I: Fig. 9), with both bent and S-shaped spores, indicated that it was a correct decision. Teratological forms appear frequently in microsporidia with rod-shaped spores, and it has e.g. been observed in Ormicresia by VIVARES, BOUIX and MANIER (1977), in Helmichia (VIII: Fig. 7), and in Mrazekia (LARSSON, unpublished).

Usually all spores are of approximately the same size, but there are species e.g. Nosema rivulogammari (IX: Fig. 24) where the spores are markedly polymorphic and of an extremely variable size. In genera with sporogony in sporophorous vesicles it is not unusual to find e.g. a small number of tetrasporous macrospores together with the usual octosporous microspores. Macrospores were e.g. revealed in Toxoglugea variabilis (I: Fig. 9), Thelohania capillata (VII: Fig. 8), Berwaldia singularis (IV), and Janacekia debaisieuxi (X). Different populations of a microsporidium may differ in spore size. This was clearly revealed by the octosporous microspores of Thelohania muelleri (IX: Figs. 9-10).

The spore wall

The plasmalemma is equally developed in all microsporidia studied. If there is an endospore, it seems invariably to be a structure-less layer, however of considerably variable thickness. The endospore is weakly developed in the primitive microsporidia. The genera Metchnikovella, Amphiacantha, Amphiamblys, and Burkea are apparently always devoid of an endospore layer, and in Hessea, Chytridiopsis and Steinhausia the layer is weakly developed or absent.

The exospore is more variable. It is usually a moderately thick electron-dense layer, e.g. as seen in Nosema tractabile (V: Fig. 15). Some species have two-layered exospores, e.g. Toxoglugea variabilis (I: Fig. 17) and Tuzetia urdae (X: Fig. 5 F). In the last mentioned species the dimensions are comparable to a unit membrane. Electron-dense material below the internal layer makes the exospore thicker in T. variabilis. In Telomyxa glugeiformis (III: Fig. 4 A) the exospore of the immature spore is two-layered, but a single dense layer in the mature spore. The exospore of Alfvenia nuda (X: Figs. 8 B, 14 B) is thick and five-layered. Spores of Thelohania and Amblyospora have thick exospores, where the surface layer, which is of homogeneous thickness, surrounds the spore completely. Below this layer is a granular or lamellar stratum, which is missing at the anterior pole (VII: Fig. 29; VI: Fig. 20).

Spores of the genus Auraspora have episporal secretions, radiating from the exospore. In Ameson michaelis the exospore is covered with fine projections, which according to DWYER and WEIDNER (1973) are microtubules. Fibres and tubules connected to the exospore are commonly found in microsporidia with sporogony in sporophorous vesicles, e.g. Thelohania and Amblyospora species.

The polar filament

In most microsporidian genera the polar filament is isofilar (IV: Fig. 4 C; V: Fig. 20). Anisofilar polar filament, with the anterior region wider than the posterior, is characteristic of the genera Agmasoma, Amblyospora (VI: Figs. 15, 18, 20), Chapmanium, Hazardia, Hyalinocysta, Parathelohania and Systemostrema. Metchnikovella and Amphibamblys have a manubrium, a short specialized polar filament. In both genera the manubrium has a wider terminal region of suggested glandular nature (VIVIER and SCHREVEL, 1973; DESPORTES and THEODORIDES, 1979). The manubrium of Baculea, Mrazekia and Ormieresia is apparently of the same construction as the normal polar filament (LOUBES and AKBARIEH, 1978; GÖTZ, 1981). The only difference is that the anterior part is straight and wide.

The internal construction of the polar filament is somewhat variable, but the fixations used are not always suitable for the preservation of the internal details. The outer lining is apparently a unit membrane (I: Fig. 12; V: Fig. 17; VI: Fig. 21). One of the numerous internal layers is usually wider and more electron-dense (III: Figs. 6 B-D; VI: Fig. 21; IX: Fig. 20), and another layer is prominently electron-translucent. The translucent layer reveals often a fibrillar structure. In Thelohania muelleri (IX: Fig. 20) 16-18 fibrils were seen, in T. capillata (VII: Fig. 32) approximately 15; in Nosema tractabile (V: Fig. 17) 18-21, and in Telomyxa glugeiformis (III: Fig. 6 B) approximately 30. The fibrillar layer of Amblyospora undulata (VI: Fig. 21) is internal to the most translucent layer. The two regions of an anisofilar polar filament are of an identical construction.

Three genera of Minisporida have peripheral specializations of the polar filament. In Chytridiopsis a honeycomb-like layer is found below the external tubulus (SPRAGUE, ORMIÈRES and MANIER, 1972). The polar filament of Steinhausia is covered with a layer of tubules, winding around the filament, which gives transverse sections a stellate appearance (RICHARDS and SHEFFIELDS, 1970). A polar filament of a similar type is found in Buxtehudea (II: Figs. 8, 11, 13).

The length of the polar filament has often been used as a diagnostic character for the species. There seems to be a considerable variation in length, although some of the short filaments possibly can be explained as incomplete extrusion. The number of polar filament coils and the length of the extruded filament are not directly corresponding. A comparison between three microsporidian species, where the spores in fixed condition are slightly longer than 3 μm , reveals the following differences. The filament of Berwaldia singularis (IV) has 15-18 coils and it is 40 μm long when extruded. In Nosema tractabile (V) there are 13-14 coils and it is 90 μm long; in Janacekia undinarum (X)

the polar filament with 7 coils is at least 90 μm long. The polar filament of J. debaisieuxi (X) is 100 μm long (MAURAND, 1975), with approximately 20 filament coils.

The coils can be arranged in a single layer, in two layers or more irregularly. The 12-14 coils of Alfvenia muda (X: Fig. 14 B) are found in two layers, while the same number of coils in Tuzetia lipotropha (X: Fig. 13 B) form a single layer. The space occupied by the coils is also variable. The coiled part in spores of Nosema tractabile begins about 1/4 from the anterior pole (V: Fig. 15), while the coils of Berwaldia singularis begin 1/3 from the front end (IV: Fig. 4 C). In the Swedish microsporidia the angle of tilt of the anterior filament coil varies from 45 to 90 degrees. A variation of 5-10 degrees is often found between different spores of a species. The usefulness of this character is not clear.

The diameter of the polar filament of Thelohania muelleri (IX) was bigger in the big spores of one of the populations, the number of coils, however, was identical in spores of both sizes.

The polaroplast

In microsporidia of the order Microsporida the polaroplast is homogeneously constructed with two lamellar parts. The anterior lamellae are usually more regularly arranged than the posterior (I: Fig. 14). However, the polaroplast lamellae are sensitive to fixation, and it cannot be excluded that irregularly arranged polaroplast lamellae are distorted, and it was e.g. seen that both polaroplast regions of Thelohania muelleri were regularly arranged (IX: Fig. 17). Some microsporidia have short anterior lamellae, forming a dense organelle. Examples are Amblyospora undulata (VI: Figs. 18, 20) and Toxogluzea variabilis (I: Fig. 14). The anterior lamellae of Nosema rivulogammari are long (IX: Fig. 46), enclosing the posterior ones in a cap-like manner.

The extension of the polaroplast varies between the species. Frequently the polaroplast occupies the anterior 1/3 of the spore, like the case of Helmuchia aggregata (VIII: Fig. 28). The polaroplast of Thelohania capillata (VII: Fig. 29) is extremely long, reaching the posterior pole of the spore.

The Metchnikovellidae are devoid of a polaroplast. Hessea has a rudimentary polaroplast (ORMIERES and SPRAGUE, 1973), in Burkea flattened Golgi vesicles are found in the same position (PUYTORAC and TOURRET, 1963; BURKE, 1970), and in Buxtehudea (II: Fig. 16) a minute lamellar structure in the anterior part of the spore is interpreted as a rudimentary polaroplast. The honeycomb-like layer of Chytridiopsis socius, and the tubules on the polar filament of Steinhausia brachynema have been suggested to be a special kind of polaroplast (VAVRA, 1976). The arrangement seen in these genera resembles primordia of a lamellar polaroplast (IX: Fig. 39).

Two microsporidian genera with rod-shaped spores have polaroplasts of unusual types. In Ornieresia the anterior polaroplast region is spongy, the posterior lamellar (VIVARES, BOUIX and MANIER, 1977). The polaroplast of Bacula is found in a lateral position to the polar filament, not concentrically enclosing it (LOUBES and AKBARIEH, 1978).

The nucleus

Most microsporidia have a single spherical nucleus in the centre of the spore, but there are also genera with a bent nucleus, e.g. Helmichia (VIII) and Metchnikovella. Nosema (V: Fig. 15) and several other genera have binucleate spores. The nuclear characteristics of the genera are listed in Table 5.

It is sometimes problematical to detect the number of nuclei. To study serial sections using electron microscopy is one method, hydrolysis of smears before staining is another. However, using the last method, the chromatin arrangement of single nuclei may give the impression of a binucleate condition, like the case of Thelohania capillata (VII: Fig. 7) and Helmichia aggregata (VIII: Fig. 4).

Centriolar plaques of variable dimensions have been observed in numerous microsporidia. They are usually seen as electron-dense areas invaginated into the nucleus (I: Fig. 7; III: Fig. 2 D; IV: Fig. 2 D; VI: Figs. 6-7; VII: Figs. 16-17; VIII: Fig. 13; IX: Figs. 35-37; X: Fig. 11 B), and most prominent in the sporont stage. There are species where the plaques are lamellar, e.g. three-layered in Glugea habrodesmi (LOUBES, MAURAND, GASC and BOUIX, 1976) and Telomyxa glugeiformis (III: Fig. 2 D). In other species, like Toxoglugea variabilis (I: Fig. 7), the plaques are homogeneously electron-dense. It is not clear what influence fixation has on the morphology of the plaques. The plaques of fungi have been studied thoroughly, and it is clear that there are different morphological types (ZICKLER, 1970). It was also found that the morphology of the plaques varied with the phase of development (HORESH, SIMCHEN and FRIEDMANN, 1979).

The posterior vacuole and the posterosomes

Many microsporidia have a membrane-lined vacuole at the posterior pole of the spore (VII: Fig. 30). The vacuole of Buxtehudea scaniae is delimited by several membranes (II: Fig. 13), an unusual condition. In other species, e.g. Telomyxa glugeiformis (III: Fig. 6 A) there is an electron-dense posterosome at the same position. In Thelohania muelleri (IX: Fig. 18) a posterosome is situated close to the vacuole. Nelliemelba boeckella has several posterosomes in addition to the vacuole (MILNER and MAYER, 1982).

The sporophorous vesicle

The sporophorous vesicle is usually a more or less persistent spherical or oval sac. Fusiform sporophorous vesicles are characteristic of the genera Amphiacantha, Amphiamblys and Chapmanium. Two genera have sporophorous vesicles with needle-like or filamentous projections: Mitoplastophora and Trichoduboscqia.

The membrane of the sporophorous vesicle is normally a thin electron-dense layer, often about 5 nm thick, not a unit membrane. A thin cover of this type is characteristic of the families Amblyosporidae (VI: Fig. 5) and Thelohaniidae (VIII: Figs. 13-14; IX: Fig. 22). The membrane of Telomyxidae is more complicated. Telomyxa glugeiformis has a membrane, which is about 20 nm thick, composed of several layers, and with a granular or fibrillar surface (III: Figs. 6 E-F). The vesicle of Berwaldia has a two-layered wall (IV: Figs. 3 D-E). Below the 5 nm thick surface layer, is a second layer composed of closely arranged tubules or fibrils. The two layers together form a vesicle wall of the same thickness as in Telomyxa. Metchnikovella, Amphiamblys, Hessea and Chytridiopsis have vesicles with thick membranes.

Fibrous material, continuous from the spore wall to the membrane of the sporophorous vesicle, is common in several genera, especially prominent in Amblyospora (VI: Fig. 8) and Thelohania (VII: Fig. 30). The fibrils of T. capillata are so strongly developed that they give the sporophorous vesicle a peculiar wavy appearance (VII: Fig. 30).

Tubules are commonly found in sporophorous vesicles, at least when they contain immature stages, and beautifully revealed using scanning electron microscopy (e.g. RAUSCH and GRUNEWALD, 1980). They are especially prominent in the Tuzetiidae, where they are either wide or narrow. The tubules of the Janacekia species are wide (X: Figs. 10 F, 14 C-D), while tubules of Tuzetia s. str. are narrow (X: Figs. 13 A-D). In the four Tuzetia species T. ecdyonuri, T. lipotropha, T. skuldae and T. verdandiae anastomosing tubules form a network below the external membrane of the sporophorous vesicle (X: Figs. 18 A-D). Toxoglugea variabilis has tubules of two dimensions (I: Fig. 19), arranged in an unusually regular manner in sporophorous vesicles with sporoblasts (I: Fig. 5). Helmichia aggregata has prominent tubules in a short period of spore morphogenesis (VIII: Fig. 25). The tubules of Thelohania muelleri appear to be septate (IX: Fig. 22).

Granular metabolic inclusions are typical of some genera with octosporous sporophorous vesicles. They are most numerous in Cryptosporina, where the spores are practically obscured by the granules (HAZARD and OLDACRE, 1975). In Amblyospora granules are numerous in vesicles with immature stages (VI: Figs. 3 D, 8), and reduced in sporophorous vesicles with mature spores (VI: Figs.

3 D, 19). The most extreme arrangement of secretory material is found in sporophorous vesicles of the Telomyxidae. The two spores of Berwaldia singularis are connected by patches of electron-dense material (IV: Figs. 4 C, E-F), and the two spores of Telomyxa glugeiformis are completely enclosed by a similar material to an oval body (III: Figs. 7 A-B). A contrary condition is found in the genus Hyalinocysta, where the sporophorous vesicles are completely devoid of secretory products.

In three species of the Tuzetiidae (X) a supplementary coat is formed below the membrane of the sporophorous vesicle. Persistent membranes of this type are found in Nelliemelba boeckella and Tuzetia sp. 1 MAURAND, 1973. A non-persistent layer is formed in the sporoblastogenesis of Janacekia undinarum (X: Fig. 12 D). This layer disappears rapidly and cannot be traced in vesicles with mature spores.

VARIATIONS IN HISTOPATHOLOGY AND HOST SPECIFICITY

Microsporidian infections are most easily detected in aquatic hosts, with more or less transparent cuticle, where the infection is revealed as an unnatural coloration. Infected hosts are generally coloured white, unusually prominent in the chalky white colour caused by Telomyxa glugeiformis (III). Yellow coloration is not unusual, seen e.g. in infections by Berwaldia singularis (IV) and Tuzetia skuldae (X). MILNER and MAYER (1982) described the coloration caused by Nelliemelba boeckella as salmon-pink or orange.

All microsporidia develop intracellularly, consuming the cytoplasm and the organelles of the host cells. A few species have been found also to invade the nuclei. TAKIZAWA, VIVIER and PETITPREZ (1973) reported intranuclear development of Nosema bombycis, PERCY (1973) of Nosema (Perezia) fumiferanae, ORMIERES, LOUBES and MAURAND (1977) of Perezia lankesteriae, SPRAGUE and VERNICK (1968) of Glugea weissenbergi, and LOUBES and AKHARIEH (1977) of Nosemoides simocephali. The microsporidia of the genus Chytridiopsis develop in close connection to the nucleus of the host cell, and are usually found in a shallow invagination of the nuclear membranes.

Infections of the gut epithelium are probably not lethal. Infected cells filled with microsporidia are often shed into the gut lumen (II: Fig. 4), and the regenerative capacity of the epithelium can cope with the infection. The gut epithelium infected with Buxtehudea scaniae is mildly disturbed. The organelles persist and the pathological changes observed are restricted to the mitochondria, which are compressed and arranged close to the vacuole with the parasites (II: Fig. 18). Infections of the organs of the haemocoel are more severe.

It has frequently been observed that the nuclei of cells infected with microsporidia are more or less hypertrophied. It is very prominent in the infection by Nosema tractabile (V: Fig. 2). There are also species, e.g. Janacekia undinarum (X: Figs. 12 A-B), which do not induce nuclear hypertrophy.

Microsporidia develop either in intimate contact with the organelles of the host cells or are enclosed by protective covers of various provenances. Nosema species develop free in the cytoplasm (IX: Fig. 29). Microsporidia of many genera produce sporophorous vesicles. Other microsporidia provoke initiation of a parasitophorous or sporogony vacuole, formed by the host cell. Buxtehudea scaniae (II: Fig. 18) and Helmichia aggregata (VIII: Figs. 12-14) develop in membrane-lined vacuoles formed by the hosts. Parasites of fish may evoke strong defence reactions, and the parasites are encapsulated by the host. Thick-walled cysts are characteristic for infections by Glugea, Loma and Spraguea, while the cyst of Ichthyosporidium has a wall of a single cell layer. Encapsulation has not been observed in invertebrates infected with microsporidia.

Infection can also induce the production of a xenoma, a functional unit of the host tissue and the parasites. The xenomas produced by the fish-parasitic Glugea species are most well-known and treated in several papers (e.g. WEISSENBERG, 1976). WEISER (1976) studied the xenoma of Janacekia debaisieuxi, and discussed the principal differences of xenomas of invertebrates, where he distinguished syncytial and neoplastic xenomas. In syncytial xenomas the membranes of the cells are dissolved, and the infected tissue is modified to a syncytium where the nuclei and other organelles of the host cells occur together with the microsporidia. Syncytial xenomas are typically produced in infections by microsporidia of the families Thelohanidae and Amblyosporidae, and seen e.g. in the infections by Thelohania capillata (VII: Fig. 1) and Amblyospora undulata (VI: Figs. 1-2). In the neoplastic xenomas the cellular nature is conserved, although the connections between individual cells may be broken. It has also been observed that the nuclei of infected cells are forced by the parasite to divide. The voluminous Glugea xenomas are the product of a single infected epithelial cell and the multiplication of the microsporidium. Neoplastic xenomas are typical of infections by the two Janacekia species, J. debaisieuxi (X: Figs. 16 A-B) and J. undinarum (X: Figs. 11 I, 16 C-D).

Different microsporidia may reveal a more or less prominent tissue affinity. There are species, e.g. Nosema tractabile (V), which can infect several tissues, while others, e.g. Buxtehudea scaniae (II), are restricted to a single tissue. Nosema brassicae and N. mesnili were originally considered as different species from differences of histopathology. N. mesnili was principally a parasite of the gut epithelium, while N. brassicae was found in the fat body and other tissues of the haemocoel. They were later merged as N. mesnili when it was found that the histopathological differences were results of different

routes of infection (MOSTOUNSKY, 1970; LARSSON, 1979). In Crustacea the musculature is the tissue preferred by the microsporidia, although other tissues can be infected too. Both Thelohania muelleri and Nosema rivuloganmari (IX) are muscle parasites. In insects the fat body is the most important site of infection, but infections of the gut epithelium are also commonly found. Of the microsporidia treated here, the following are parasites of the fat body: Oxogluges variabilis (I), Telomyxa glugeiformis (III), Amblyospora undulata (VI), Thelohania capillata (VII), Helmichia aggregata (VIII), Tuzetia ecdyomuri, T. lipotropha, T. urdae, T. verdandiae, Janacekia debaisieuxi and J. undinarum (X). Two species were exclusively found in the gut epithelium: Buxtehudea scaniae (II) and Tuzetia skuldae (X).

The information on the host specificity of different microsporidian species is meagre. The microsporidia are not seldom considered to be host specific, and there are numerous species described, where the only distinctive character is the occurrence in a previously unknown host. Although it seems plausible that a microsporidian species could be capable to develop at least in closely related hosts, there are apparently various grades of host specificity. Nosema bombycis seems to have a wide host spectrum, and successful inoculations have been made to a great number of lepidopteran species of widely separated families. Telomyxa glugeiformis (III) has apparently a narrow host range. It has only been reported from the two species Ephemera vulgata and E. danica. Since numerous microsporidia are known from the Ephemeroptera, and this microsporidium is so characteristic that it cannot be confused with any other species, neither can the prominent infection be overlooked, it should have been observed if the species occurred also in other hosts.

The microsporidia of this thesis have not been inoculated into other hosts to determine the host range. However, collecting has always been performed in such way that samples of the total fauna were brought into the laboratory, where all infected specimens were sorted out. If infection was found in one host species, related hosts were carefully checked for infections, see e.g. discussions on Nosema tractabile (V: 417) and Berwaldia singularis (IV: 337). If microsporidia are found in hosts living in small ponds, there should have been great opportunities for other susceptible hosts in the same pond to acquire infection.

VARIATIONS IN THE DEVELOPMENTAL CYCLES

Schizogony is important in the protozoan phyla with exclusively parasitic species, also in the Microspora. Merogony and sporogony are regularly present

in the development, but gamogony is so far unknown to occur. The terminology of schizogony recommended by LEVINE (1971) for the Apicomplexa is also followed here.

While sporogony is known to occur in the development of all microsporidia, there are a few groups where merogony has not been observed, and is presumed to be absent. The primitive microsporidia of the Hetchnikovellidae, Chytridiopsidae, Durkeidae and Buxteludeidae (II) seem to have no merogonial reproduction.

In the order Microsporida there are several taxa where merogony has not been observed. This can usually be explained by the fact that the microsporidium was studied in material directly collected from the nature, where the microsporidia of hosts with prominent infections usually are in the sporogonial phase of development. In the genus Nosema, with numerous species, there seems to be species differences concerning the importance of merogony in the development. Merogony was not revealed in Noscoma tractabile (V), one sequence was observed in N. rivulogammari (IX), and GRAY, CALI and BRIGGS (1969) reported two merogonies in the development of N. apis. There are numerous reports of Nosema species, describing these three varieties.

If there are two merogonies, micronuclear merozoites are often produced by the first merogony and macronuclear by the second, like the case of Berwaldia singularis (IV: Figs. 1 C-D). The merozoites of the Nosema species are usually produced in chains, e.g. seen in N. rivulogammari (IX: Fig. 29). Merozoites can also be formed by rosette-like division of plurinucleate plasmodia, like the first merogony of Berwaldia singularis (IV: Fig. 1 C).

The reproductive capacity of the sporogony is variable. In genera where merogony is supposed to be absent, e.g. Buxteludea (II), sporogony is the only way of reproduction, and it results usually in the production of numerous spores. In other genera, for example Nosema (V), Berwaldia (IV) and Telomyxa (III), only two spores are produced by each sporont. In most microsporidian genera the number of sporoblasts produced by each sporont is fairly constant, at least in the interval 2-32 (Table 4).

The sporoblasts are either formed from a sporogonial plasmodium, where all sporoblasts are set free at the same time, e.g. as in Thelohania capillata (VII: Fig. 20), or the sporoblasts are produced by subsequent divisions. In the development of Amblyospora undulata (VI) the 8 sporoblasts are either formed simultaneously from a single division, or by two divisions where the first results in four cells, each of which divides into two sporoblasts. The sporogony of Janacekia undinarum (X) is also interpreted to proceed in two steps.

TABLE 4. The number of sporoblasts produced by each sporont

F: genus with free spores; SV: genus with spores produced in a sporophorous vesicle

MONOMORPHIC SPORULATION			DIMORPHIC SPORULATION	
			I. With nuclear dimorphism	
2	Bervaldia SV	16	22/2-16	Hazardia F/F
	Cougourdella F	32	8/2	Purenella SV/F
	Eucephalitozoon F	n		Vairimorpha SV/F
	Glugea SV		8/n	Amblyospora SV/F
	Hessea SV		n/?	Parathelohania SV/F
	Ichthyosporidium F			Aurasporea SV/F
	Issia SV			
	Loma F			
	Mrazekia F			
	Neoperezia SV			
	Nosema F			
	Oligosporidium F			
	Telomyxa SV			
	Unikaryon F			
4	Gurleya SV			
	Pyrotheca SV			
8	Agmasoma SV			
	Ceudosporea F	2 - 4		
	Chapmanium SV	2 - 8		
	Cryptosporina SV	4 - 8		
	Helmichia SV	4 - 16		
	Hyalinocysta SV	5 - 6		
	Indosporus SV	6 - 8		
	Octosporea SV	8 - 12		
	Ormieresia SV	8 - 16		
	Pegmatheca SV	8, n		
	Pilosoporella SV	12 - n		
	Polydispyrenia SV	16 - 25		
	Systenostrema SV	16 - 32		
	Thelohania SV	?		
	Toxoglugea SV	?		
			II. Without nuclear dimorphism	
			4/8	Stempellia SV/F

Most microsporidian genera have monomorphic sporogony, i.e. all spores produced are of the same type. It is not unusual that an octosporous microsporidium produces a small number of tetrasporous macrospores, e.g. seen in the development of Toxoglugea variabilis (I: Fig. 9) and Thelohania capillata (VII: Fig. 8). This is also a case of monomorphic sporogony, for the macrospores produced do not differ from the microspores, i.e. the normal spores, in essential characters. They are a little bigger and have usually supernumerary polar filament coils (VII: Figs. 33, 35). Other genera show dimorphic sporogony (Tables 4 and 5). The spores are produced by two different processes, and the spores formed are of different qualities, e.g. concerning the number of nuclei. In the dimorphic genera it is usual that one sporogony produces uninucleate spores in sporophorous vesicles, while the other sequence yields free diplokaryotic spores. This is characteristic of the genera Durenella, Vairimorpha, Amblyospora and Parathelohania. In Hazardia both sequences yield free spores, uninucleate in one sequence, binucleate in the other. In the genera Durenella, Vairimorpha and Hazardia both sporogonies occur together in the same host specimen. In Amblyospora and Parathelohania binucleate free spores are found in adult females, and octospores with single nuclei in male larvae. However, there are species of the genus Amblyospora where both free spores and octospores are found together in larval specimens. HAZARD and OLDACRE (1975) observed the two spore types in preparations of A. callosa, but were not certain that they represented two sporogonial sequences. The same condition was observed in A. callosa collected in Sweden (LARSSON, 1983), and the two spore types were ultrastructurally identical to the spores of A. callosa from the U.S.A. The same phenomenon has also been recorded in other Swedish species of Amblyospora (LARSSON, unpublished).

In the genus Stempellia both sporogonies yield uninucleate spores, free in one sequence, enclosed in tetrasporous sporophorous vesicles in the other.

All developmental stages of a species may be uni- or binucleate, or uni- and binucleate stages alternate. Berwaldia singularis (IV) is uninucleate in all stages, while the species of Nosema (V, IX) are binucleate. In the genera Amblyospora (VI) and Thelohania (VII, IX) merogonial stages and sporonts are diplokaryotic, but the spores are uninucleate. In Culicospora the meront is diplokaryotic, while the sporont and spore are uninucleate. The nuclear conditions of the microsporidian genera are summarized in Table 5.

It is well-known that microsporidia of several genera have meiotically dividing sporonts. The first reports were published in 1976 (DESPORTES; LOUBES and MAURAND; VAVRA), all based on the observations of synaptonemal complexes in the dividing nucleus of the sporont. There are also investigations where the different phases of meiosis have been studied using light microscopy (HAZARD, ANDREADIS, JOSLYN and ELLIS, 1979). LOUBES (1979) published a

Meront 2 - Sporont 1 - Spo

[illegible]

detailed treatise on the meiosis of microsporidia. The occurrence of meiosis has been revealed in the following genera: by LOUBES and MAURAND (1976) in Gurleya, by DESPORTES (1976) in Stempellia, by VAVRA (1976) in Janacekia, by LOUBES and AKDARIEH (1976) in Baculea, by HAZARD, ANDREADIS, JOSLYN and ELLIS (1979) in Amblyospora and Parathelohania, by LOUBES (1979) in Polydictyonella, and by MALONE (1982) in Vairimorpha. LOUBES (1979) reported meiosis to occur in Thelohania fibrata, which according to a micrograph in a paper by MALONE and LOUBES (1978) is an Amblyospora species, and in Thelohania sp. from the midge Cricotopus. Since there are no illustrations of the spore it is not clear if this is a Thelohania or an Amblyospora species. Meiosis was observed in the following Swedish microsporidia: Toxoglugea variabilis (LARSSON, unpublished), Thelohania capillata (VII: Fig. 19), Helmichia aggregata (VIII: Figs. 17, 19), and Janacekia undinarum (X: Fig. 11 C). Structures resembling synaptonemal complexes have been observed in the sporont nuclei of Telomyxa glugeiformis (III: Fig. 2 D) and Nosema rivulogammari (IX: Figs. 32-33). Both uninucleate and diplokaryotic sporonts have been found to divide meiotically.

Of the 69 microsporidian genera meiosis has been recorded in 11, and it is suspected to occur in some more. Further investigations are necessary to reveal if meiosis is an overlooked general phenomenon in microsporidian development or a specialisation restricted to a small number of microsporidian species or genera.

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APPENDIX I

DICHOTOMOUS IDENTIFICATION KEY TO THE GENERA OF MICROSPORIDIA

* indicates that the genus is insufficiently known

1. Spores glued together pair-wise in a common matrix to an oval body *THLOPAXA*
 - Spores not embedded in a common matrix 2
2. Exospore with 1-5 tail-like appendages 3
 - Exospore without tail-like appendages 4
3. Spores uninucleate, with 4-5 appendages, in octosporous sporophorous vesicles *INCOUSIDIA*
 - Spores free, with 1 tail-like prolongation 4
4. Spores rod-like, uninucleate; parasites of Oligochaeta *JIROVECTIA*
 - Spores oval, binucleate; parasites of Simuliidae *CAUDOSPORIA*
5. All spores rod-like, cylindrical; straight, bent or spiralized 6
 - Spores not rod-shaped or rod-shaped spores occur together with oval spores 12
6. Spores C-shaped or spiralized *TOXOGLUGEA*
 - Spores straight or lightly bent 7
7. Spores in octosporous sporophorous vesicles or in multisporeous thick-walled cysts 8
 - Spores free but may occur in vacuoles 11
8. Thick-walled cysts with 32 spores; spores with a manubrium; parasites of Gregarinida *AMPHIAMBLYS*
 - Spores in octosporous sporophorous vesicles 9
9. Spores binucleate; 3-10 μm long *OCTOSPOREA*
 - Spores uninucleate 10
10. Spores more than 16 μm long; with a manubrium, two polar rings and a spongy anterior polaroplast region; parasites of Crustacea *ORNITHOSIA*
 - Spores not longer than 7 μm ; without manubrium and polar rings, both polaroplast regions lamellar; parasites of Insecta *HELMICHTIA*
11. Spores with a posterior swelling; sporulation in direct contact with the cytoplasm; disporoblastic **COUGOURDELLA*
 - Spores not wider posteriorly 14
12. Spores usually more than 15 μm long; binucleate, ungrouped; usually parasites of Oligochaeta *MRAZELIA*
 - Spores not longer than 5 μm ; uninucleate, in multisporeous groups in vacuoles; parasites of Crustacea *BACULEA*
13. Oval and rod-shaped spores occur together, both types uninucleate; parasites of Gregarinida *NOSEMOIDES*
 - No rod-shaped spores 14
14. Spores spherical or lenticular 15
 - Spores of a different shape 24
15. Spores uninucleate, in octosporous sporophorous vesicles. *PILOSPORELLA*
 - Spores free or in multisporeous sporophorous vesicles or cysts 16

16. Parasites of Gregarinida; spores without polaroplast and endospore;
sporulation in a thick-walled cyst and a vacuole 17
- Not parasites of Gregarinida; polaroplast and endospore variable .. 19
17. Sporophorous vesicle (cyst) with pointed ends *AMPHIACANTHA
- Sporophorous vesicle (cyst) with rounded ends 18
18. Sporophorous vesicle (cyst) longer than 10 x the diameter, AMPHIAMBLYS
- Sporophorous vesicle (cyst) shorter than 10 x the diameter METCHNIKOVELLA
19. Microsporidia develop in close connection to the nucleus of the host
cell 20
- Microsporidia not prominently associated with the nucleus of the host
cell 21
20. Parasites of Mollusca; especially in the gut epithelium and the ova-
ries; polar filament with an external honeycomb-like layer. STEINHAUSIA
- Parasites of Insecta and Myriapoda; in the gut epithelium; polar fila-
ment of the usual type CHYTRIDIOPSIS
21. Meront and sporont diplokaryotic; in sporophorous vesicles ornamented
with polygonal plaques; in the gut epithelium of Insecta HESSEA
- No diplokarya; no sporogonial vesicles 22
22. In muscles and epidermis of Annelida; infected cells hypertrophied,
developing into xenomas BURKEA
- Without production of xenomas 23
23. In the gut epithelium of Insecta BUXTEHUDEA
- In the gut epithelium of Oligochaeta *JIROVECIANA
24. Spores font-like flattened at one side and at the anterior pole or
exospore with prominent ridges and sutures 25
- Spores of various shapes, but without unilateral depressions or
ridges 26
25. Spores with one side and the anterior pole flattened, binucleate
..... *GCLBERGIA
- Spores with prominent ridges WEISBERIA
26. Sporophorous vesicles with needle-like or flagelliform appendages.. 27
- Spores free or in sporophorous vesicles without appendages 28
27. Fresh sporophorous vesicles spherical, with 4 needle-like appendages;
most sporophorous vesicles with 16 or 32 spores TRICHODUBOSCQIA
- Sporophorous vesicles fusiform or triangular, with the angles prolona-
ted by 2-3 flagelliform filaments; sporophorous vesicles with 2-64
spores *MITOPLISTOPHORA
28. Parasites of fish, producing voluminous xenomas 29
- Parasites of various hosts, if parasites of fish no production of
xenomas 32
29. Each xenoma formed from a single host cell; cyst wall thick; nucleus
of the host cell divides or is strongly lobed; spore uninucleate .. 30
- Each xenoma formed from several host cells; cyst wall thick or thin;
nuclei of host cells undisturbed; spores uni- or binucleate 31
30. Sporophorous vesicles with numerous spores; immature stages at the
periphery of the xenoma GLUGEA
- No sporophorous vesicles; mature and immature stages randomly dis-
persed in the xenoma LCMA

31. Xenoma with a thick cyst-wall; in nerve tissue; spores uninucleate	31
- Xenoma with a thin cyst wall; in connective tissue of the abdomen; spores binucleate	32
32. Spores connected pair-wise	33
- Spores not connected pair-wise	34
33. Spores binucleate	35
- Spores uninucleate	36
34. Sporophorous vesicles wide, membrane not attached to the surface of the spores; merozoites diplokaryotic	37
- Sporophorous vesicles with folded membrane, attached spot-wise to the surface of the spores, inner layer of the membrane tubular; all stages uninucleate	38
35. Sporophorous vesicles with 4 spores of an identical size	39
- Spores free or differently grouped	40
36. Spores elongated pyriform, with a bent apical part; parasites of Crustacea	41
- Spores pyriform; parasites of various invertebrates	42
37. Free binucleate macrospores occur together with uninucleate microspores, which usually are enclosed in sporophorous vesicles	43
- All spores either binucleate or uninucleate	44
38. Uninucleate spores in octosporous sporophorous vesicles	45
- Uninucleate pyriform spores produced in an irregular number	46
39. Octospores with an anisofilar polar filament; sporophorous vesicles usually with metabolic granules	47
- Polar filament isofilar; sporophorous vesicles without granules ..	48
40. Sporophorous vesicles with a thick, persistent wall; polar filament in a single layer close to the spore wall	49
- Sporophorous vesicles with a fragile membrane; polar filament coils irregularly arranged	50
41. Spores without prominent episporal secretions; parasites of Culicidae	51
- Spores with foamy episporal secretions; parasites of Collembola	52
42. All or a part of the spores enclosed in sporophorous vesicles	53
- All spores free	54
43. Macrospores in tetrasporous sporophorous vesicles, microspores free	55
- Spores in sporophorous vesicles with 1, 2, 4, 8 or more spores	56
44. Each sporophorous vesicle with a single spore	57
- Sporophorous vesicles at least with 2 spores	58
45. Polar filament anisofilar	59
- Polar filament isofilar	60
46. Meront and sporont uninucleate	61
- Meront and sporont diplokaryotic	62
47. Exospore thick, multilayered; sporophorous vesicle without tubules; parasites of Crustacea	63
- Exospore thin, homogeneous; sporophorous vesicle with wide tubules; parasites of Simuliidae	64

40. Sporophorous vesicles with a variable number of spores, all spores of a uniform size; parasites of Culicidae 49
- Sporophorous vesicles with 8, 16, 32 or more spores; a small number of tetrasporous sporophorous vesicles with macrospores can occur together with octosporous microspores or a small number of octosporous macrospores are found together with multisporeous microspores 50
41. Spores 12-16 μm ; sporophorous vesicles with 4, 8 and 16 spores occur together *CULICOSPORA
- Spores smaller than 6 μm ; sporophorous vesicles with 8, 16 and 32 spores occur together VAVRAIA
42. Sporophorous vesicles with 5-6 binucleate spores; parasites of Insecta *CULICOSPORELLA
- Sporophorous vesicles with more spores 51
43. Sporophorous vesicles octosporous 52
- Sporophorous vesicles with more spores 61
44. Polar filament anisofilar 53
- Polar filament isofilar 58
45. Sporophorous vesicles fusiform, spores pyriform CHAPMANIUM
- Sporophorous vesicles spherical or oval 54
46. Sporophorous vesicles without granular inclusions 55
- Sporophorous vesicles with granular inclusions 56
47. Sporophorous vesicles oval, the unstained vesicles are prominent in stained smears; spores usually pyriform with a big lamellar polaroplast; parasites of Insecta HYALINOCYSTA
- Sporophorous vesicles spherical-oval; spores pyriform with an indistinct polaroplast; parasites of Crustacea AGMASOMA
48. Octospores with a constricted posterior end PARATHELOHANIA
- Octospores oval-pyriform, not constricted posteriorly 57
49. Spores pyriform SYSTEINOSTREMA
- Spores oval-barrelshaped AMBLYOSPORCA
50. Parasites of fish; a small number of sporophorous vesicles with 16 spores can occur together with octosporous sporophorous vesicles *HETEROSPORIS
- Parasites of invertebrates; a small number of sporophorous vesicles with 2 or 4 macrospores can occur together with octosporous microspores 59
51. Sporophorous vesicles oval, with numerous amber-coloured granules, nearly obscuring the spores CRYPTOSPORA
- Sporophorous vesicles of various shapes; granules not so numerous that they obscure the spores 60
60. Sporophorous vesicles connected by cytoplasmic bridges until sporulation has terminated; sporophorous vesicles formed by plurinucleate plasmodia PEGMATHECA
- Sporophorous vesicles not connected THELOHANIA
61. Sporophorous vesicles with 16 spores; the wall without filamentous appendages DUBOSCQIA
- Sporophorous vesicles with a different number of spores 62
62. Spores binucleate; sporophorous vesicles with numerous spores; finger-like protrusions from the sporogonial plasmodium penetrate the cytoplasm of the host cell; parasites of Polychaeta .. *PSEUDOPLEISTOPHORA
- Spores uninucleate 63

63. Meront and sporont diplokaryotic; sporophorous vesicles with numerous spores; octosporoblastic; no macrospores; parasites of Insecta POLYSPOROBlastic
- Meront and sporont uninucleate; two kinds of sporophorous vesicles, with numerous microspores and with 8 macrospores; parasites of fish and amphibia POLYSPOROBlastic
64. Spores birucleate POLYSPOROBlastic
- Spores uninucleate POLYSPOROBlastic
65. Sporont disporoblastic POLYSPOROBlastic
- Sporont polysporoblastic POLYSPOROBlastic
66. Spores with tubular episporal secretions; parasites of Crustacea POLYSPOROBlastic
- Spores without episporal secretions; in various hosts, especially adult female Diptera AMELYOSPORE and PARATHELOMANIA
67. Sporont disporoblastic POLYSPOROBlastic
- Sporont polysporoblastic; parasites of Gregarinida 70
68. Parasites of vertebrates POLYSPOROBlastic
- Parasites of invertebrates POLYSPOROBlastic
69. Sporulation in parasitophorous vacuoles; parasites of Arachnida ... POLYSPOROBlastic
- No parasitophorous vacuoles; parasites of Trematoda UNIKARYON
70. Spores pyriform; 8-12 sporoblasts; in parasitophorous vacuoles ... POLYSPOROBlastic
- Spores oval; without parasitophorous vacuoles 71
71. Polysporoblastic; sporoblasts formed from moniliform plasmodia, PEREZIA
- Sporoblasts 6-8 GEUSIA

